

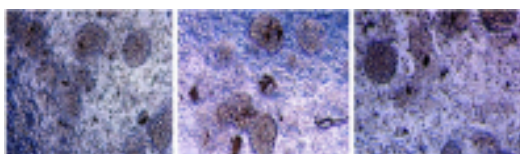
# Inhibition of HIV cell-to-cell fusion by antiretroviral drugs and neutralizing antibodies

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## Introduction

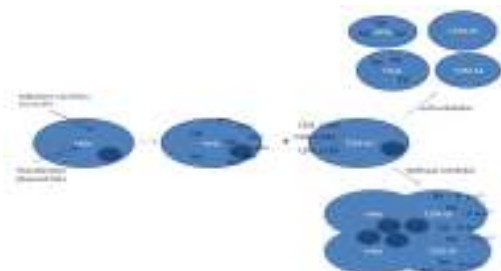
Inhibition of HIV cell entry by antiretroviral drugs and neutralizing antibodies (NABs) is typically measured in assays where cell-free virions enter reporter cell lines. However, direct Env-mediated cell-to-cell transmission is a major mechanism of HIV infection that also needs to be targeted. In this work we aimed to determine the ability of anti-HIV compounds in clinical or research use to inhibit HIV mediated cell-to-cell fusion (syncytia formation).



**Figure 1** -Microscopic observation of syncytia for untreated HeLa and TZM-bl cells. (magnification:100X)

## Methods

We developed a new method in which HeLa-CD4- cells are first transfected with a Tat expressing plasmid (pcDNA3.1+Tat101) and infected with recombinant vaccinia viruses expressing either the HIV-1 (vPE16) or HIV-2 (vSC50) envelope glycoproteins (M.O.I.=1 PFU/cell). The cells are then added to TZM-bl cells (express the CD4, CCR5 and CCR4 receptors and luciferase) in the presence of the drugs under analysis at different concentrations. When cell-to-cell fusion (syncytia) occurs the Tat protein diffuses to the TZM-bl cells activating the expression of a reporter gene (luciferase).

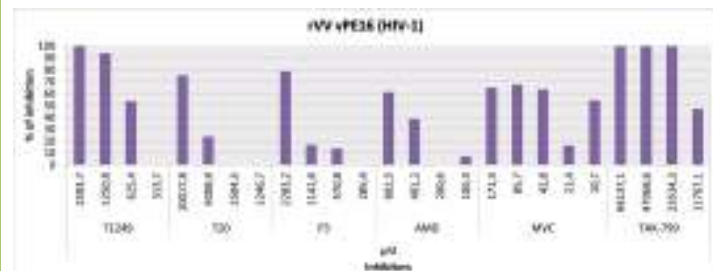


**Figure 2** - Schematic representation of the novel method used to test cell-to-cell fusion inhibition

## Results

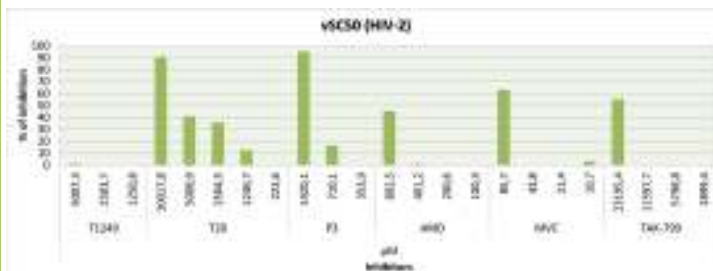
We tested several entry inhibitors including the fusion inhibitors T1249, T20 and P3, the CCR5 antagonists Maraviroc and TAK-779, the CXCR4 antagonist AMD3100 and several neutralizing antibodies. All compounds inhibited HIV-1 and HIV-2 cell fusion albeit to different levels.

For HIV-1, the best cell fusion inhibitor was Maraviroc with an IC<sub>50</sub> of 0.0076μM. T1249 and P3 had IC<sub>50</sub>s of 0.61μM and 1.34μM, respectively. TAK-779 was the weakest inhibitor, with an IC<sub>50</sub> of 12.64μM. Maximum percentage of inhibition (MPI) by T20 was 75% at 10.02μM and by AMD3100 was 61% at 0.80μM. We are currently testing reference NABs from HIV-1 infected patients for their cell fusion inhibition activity.



**Figure 3** - % of inhibition of rVV vPE16 (HIV-1) with different concentrations of inhibitors.

For HIV-2, Maraviroc was also the best cell fusion inhibitor (IC<sub>50</sub>= 0.0603 μM) and T20 the worst (IC<sub>50</sub> of 3.86μM). MPI by P3 was 95% at 1.42 μM, by AMD3100 was 45% at 0.80μM, by T1249 was 99.8% at 5μM and by TAK-779 was 55% at 23.20μM. The NABs from HIV-2 infected patients we have tested so far did not prevent cell fusion.



**Figure 4** - Percentage of inhibition of vSC50 (HIV-2) with different concentrations of inhibitors

Cell fusion inhibition requires higher concentrations of inhibitors for HIV-1 and HIV-2, than cell free infection.

**Table 1** - IC<sub>50</sub> values for HIV-1 and HIV-2 cell free and cell fusion inhibition of infection

Inhibitor	Cell free IC <sub>50</sub> (μM)		Cell fusion IC <sub>50</sub> (μM)		Ratio cell fusion IC <sub>50</sub> /cell free IC <sub>50</sub>	
	HIV-1	HIV-2	HIV-1	HIV-2	HIV-1	HIV-2
T1249	0.0010	0.0040	0.6121	-	306.1	-
T20	0.0012	0.1815	-	3.8602	-	13.7
P3	0.0118	0.0638	-	-	112.4	-
AMD3100	0.0021	0.0026	-	-	-	-
MVC	0.0017	0.0021	0.0026	0.0603	4.4	18.7
TAK-779	0.0233	0.0199	12.6407	-	540.5	-

## Conclusions

- HIV replicates more efficiently and rapidly through direct contact between cells, and this mode of transmission likely mediates a significant fraction of viral spread and immune evasion *in vivo*.
- This form of dissemination appears to be less susceptible to inhibition by antiretroviral drugs than cell-free virus transmission.
- Fusion and entry inhibitors in clinical use are much more effective at preventing cell-associated HIV-1 entry than HIV-2.
- Our new method will be useful to quickly identify new drugs and antibodies that can prevent cell-to-cell HIV-1 and HIV-2 infection.

## Acknowledgements

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